SUPPLEMENTARY DISCUSSION

Sources of glutamate release in the IPL

We assumed that BC axon terminals are the main source of glutamate release in the IPL. However, ectopic synapses along the axons of some On BCs[1,2] may also contribute to the observed On responses in the Off sub-lamina (Extended Data Fig. 5a,g). Two findings strongly argue against this possibility: First, these responses were always accompanied by a dominant Off component (Extended Data Fig. 5a, Fig. 2e) and second, were delayed relative to On responses in the On layer (Extended Data Fig. 5c). Instead, they likely either resulted from surround inhibition or from intrinsic properties of some Off BC types, such as a depolarising rebound following transient inhibition[3,4]. Notably, such delayed On events were also observed in some Off-type RGCs[5].

In addition, there is at least one type of glutamatergic AC (GAC) in the mouse retina, which stratifies between the On and Off ChAT bands and receives inputs from both On and Off BCs[6]. Dendritic calcium imaging suggests that GACs process BC input locally, such that GACs are expected to provide separate On and Off glutamate output signals in the On and Off sub-lamina, respectively[7]. Because GAC signals are therefore probably similar to the respective BC inputs, it is unlikely that we can separate the two using our clustering method and decided against including the GAC in our analysis.

Recently, Della Santina et al.[8] discovered a glutamatergic monopolar interneuron (GluMI) that stratifies similarly to CBC type 2 but lacks any dendrites and thus direct excitatory input from photoreceptor (discussed in ref.[9]; see also ref.[10]). Electrophysiologically, GluMIs display different light responses compared to CBC type 2[8]. To test if we could extract their functional glutamate signature using our clustering analysis, we included one additional IPL profile into our clustering (Methods). We found that ROIs in the outer-most IPL stratum could indeed be divided into three distinct response types (Extended Data Fig. 10). One of these clusters was supressed during light stimulation relative to baseline, potentially consistent with electrophysiological recordings from GluMIs[8]. However, because so far little is known about this cell type, we decided against including GluMIs in our main analysis.

In contrast, we included RBCs as they displayed robust light-evoked calcium responses at our stimulus intensities in the low-photopic regime (Extended Data Fig. 2g-j, Extended Data Fig. 4k-n). Under these conditions, rod photoreceptors, which provide the excitatory synaptic input to RBCs[11–13] are thought to be saturated. However, recent evidence suggests that both rods[14] and RBCs[15] can be active under photopic conditions. Perhaps direct contacts between RBCs and cones identified at both the ultrastructural[16] and functional level[17] contribute to the observed responses, further challenging the view that RBCs solely mediate vision in dim light.

ROI detection and verification

We defined individual glutamate “release units” based on local image correlation (Extended Data Fig. 1), resulting in 74±24 ROIs per scan field (Extended Data Fig. 1h). To verify the performance of this algorithm, we used calcium imaging of BCs with the GCaMP6f biosensor[18], where individual terminal systems (the total of all axon terminals of a single cell) as well as single axon terminals (a presynaptic varicosity) could be easily resolved (Extended Data Fig. 2a). Our algorithm reliably detected individual axon terminals and rather assigned two ROIs to a single terminal before merging two terminals into one ROI (Extended

Data Fig. 1g, Extended Data Fig. 2c). In addition, receptive field sizes estimated from calcium signals of single terminals closely fit those estimated from single iGluSnFR ROIs (Extended Data Fig. 2f) and matched the anatomical dimensions of BC dendritic fields[16,19]. Accordingly, each ROI likely captured the light-driven glutamate signal of at most one individual BC axon terminal.

Anatomical verification of clustering

Since the stratification profiles of some BC types are highly overlapping (e.g. CBC1 vs. 2), our functional BC clusters cannot unambiguously be matched to anatomical types in a one-to-one fashion. We think, however, that a 1:1 type-cluster match is secondary, as long as individual clusters accurately reflect the functional signature of individual BC types. To test if this is the case, we injected individual BCs with a red fluorescent dye before performing glutamate imaging (Extended Data Fig. 4). This allowed us to correlate an individual cell´s anatomy, including its stratification profile, with the assigned functional clusters of all of its individual terminals.

We found that in the majority of cases, ROIs assigned to individually labelled cells were allocated to the same cluster (Extended Data Fig. 4b,k). Also, the stratification profiles of the reconstructed cells matched the profiles of the respective BC types underlying the assigned functional cluster (Extended Data Fig. 4c,h,l). In a few cases, however, ROIs of the same cell were allocated to two different functionally very similar clusters (e.g. C_{3a} and C_{3b}, Extended Data Fig. 4g). When not discriminating between the clusters in these cases (C_{3a} vs. C_{3b}; C_{5i}, C_{5o} vs. C_{5t}), our clustering approach assigned 90% of all ROIs from one reconstructed BC to the same functional cluster (Extended Data Fig. 4o, bottom); when considering all clusters as separate, the performance was slightly lower (81%; Extended Data Fig. 4o, top).

The dataset for the injected BCs is considerably noisier than our main dataset as (i) we recorded wherever we could successfully inject a cell and not necessarily where the glutamate signal was best, and (ii) we used a 32x32 pixel scan configuration to better capture the labelled axon terminal system, leading to a lower sampling rate (15.625 Hz instead of 31.25 Hz). Therefore, the cluster assignments from these data were less precise, meaning that the reported fraction of “correctly” assigned ROIs likely represents a lower bound on the accuracy of our clustering approach.

The elementary computational unit of the IPL

The powerful effect of inner retinal inhibition on visual encoding in BCs bolsters the view that the elementary computational unit of the IPL must be the individual BC synaptic terminal[20–24]. Clearly, BCs are not electrotonically compact units, where the computational output can be assessed equally in each compartment[25]. Instead, individual terminals of a single BC may signal independently. To what extent such heterogeneity at the level of the BC axon terminal (“presynaptic multiplexing”) matters is actively debated[26]. While direct evidence for heterogeneity at the level of mouse BC terminal calcium is lacking[27,28], electrical recordings in salamander retina showed that individual BCs can elicit different responses in different postsynaptic RGCs[29,30]. Although this effect could be explained by presynaptic heterogeneity generated by differential AC inputs or by differences in terminal size[31], it could also originate from selective postsynaptic inhibition[32]. Additional diversity could come from type-specific differences in the dendritic properties of postsynaptic neurons[33–35]. For our recording conditions, type-specific functional differences across mouse BCs appear to be more

pronounced than any putative differences across terminals of a single cell. However, residual variability present in individual BC clusters might be explained by multiplexing.

Pharmacological dissection of AC effects on BC output

In our pharmacological experiments, we probed the dominant influence of the two main sub-classes of AC – small-field glycinergic and large-field GABAergic cells – on BC output in the whole-mounted retina. We found that glycinergic ACs primarily modulate BC output in an indirect way by inhibiting GABAergic ACs. Upon blocking glycine receptors, this network effect resulted in a surround strength which exceeded the centre strength and consistently induced a polarity switch in BC responses to full-field stimulation. In the absence of drugs, a polarity switch could also be consistently induced by presenting an annulus chirp that excluded the central 100 µm-spot (Extended Data Fig. 6f) (see e.g. ref.[36]). Accordingly, the BC surround not only modulates an existing centre response, but can also independently drive glutamate release from BCs. One explanation is that inhibition modulates a light-independent tonic release of glutamate, which appears to be a common feature of BCs[37].

Additionally, we found that blocking GABA receptors increased the size of the BC RF centre, whereas glycine receptor block had no detectable effect on RF size (Extended Data Fig. 6g,h). This implies that not only temporal, but also spatial properties of the BC centre depend on the state of the GABAergic inhibitory network in the inner retina.

Under our experimental conditions (i.e. whole-mounted retina, adaptation state and low-photopic visual stimulation; cf. Methods), glycinergic effects via serial pathways – such as the gating of GABAergic inhibition to BCs – were more pronounced than direct glycinergic effects on BCs, such as in the case of crossover inhibition (Extended Data Fig. 7). While such serial inputs have been demonstrated in salamander[38,39], available data for the mouse is less conclusive[40,41]. Earlier studies had implicated glycinergic ACs mainly in vertical signalling like crossover inhibition[24,42–44]. However, these studies were performed in vertical slices where lateral connections are likely compromised.

The origin of BC functional diversity

We found that functional diversity amongst BCs is primarily driven by a change in the ratio of excitation and GABAergic inhibition, which in turn is set by glycinergic inhibition (cf. previous section). Specifically, GABAergic inhibition seems to have distinct effects on different BC types and can thus act to decorrelate BC channels (Figs. 3-5).

We think that the observed decorrelating surround effects were mainly due to AC mediated inhibition in the inner retina – rather than horizontal cell (HC) mediated inhibition in the outer retina – for two reasons: (*i*) We found that the GABAergic BC surround was strongly gated by glycinergic signals (Fig. 4, Extended Data Fig. 6), which argues against a large GABA-mediated HC component, as glycine receptors are exclusive to the inner retina[45]. (*ii*) Our results are in line with earlier findings that mouse HCs[46] appear to have little effect on the RF structure of BCs(ref.[47], but see ref.[48]) and RGCs[49–51].

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